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Animal models of rheumatoid arthritis

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Abstract

Animal models of arthritis are used to study pathogenesis of disease and to evaluate potential anti-arthritic drugs for clinical use. Therefore morphological similarities to human disease and capacity of the model to predict efficacy in humans are important criteria in model selection. Animal models of rheumatoid arthritis (RA) with a proven track record of predictability for efficacy in humans include: rat adjuvant arthritis, rat type II collagen arthritis, mouse type II collagen arthritis and antigen-induced arthritis in several species. Agents currently in clinical use (or trials) that are active in these models include: corticosteroids, methotrexate, nonsteroidal anti-inflammatory drugs, cyclosporin A, leflunomide interleukin-1 receptor antagonist (IL-1ra) and soluble TNF receptors. For some of these agents, the models also predict that toxicities seen at higher doses for prolonged dosing periods would preclude dosing in humans at levels that might provide disease modifying effects. Data, conduct and features of the various models of these commonly utilized models of RA as well as some transgenic mouse models and less commonly utilized rodent models will be discussed with emphasis on their similarities to human disease.

Keywords: Rheumatoid Arthritis, Adjuvant Arthritis, Type II Collagen Arthritis, Antigen Arthritis, Methotrexate, Dexamethasone, Indomethacin, IL-1ra, TNF-RI

Introduction

Animal models of rheumatoid arthritis are used extensively in research on pathogenesis of inflammatory arthritis and in the pharmaceutical industry in the testing of potential anti-arthritic agents. Important criteria in selection of a model include 1) capacity to predict efficacy of agents in humans, 2) ease of performing the model, reproducibility of data, reasonable duration of test period and 3) similar pathology and/or pathogenesis to that of human disease. In the area of rheumatoid arthritis, there are excellent models that have good track records for predictability. This is in large part due to the fact that numerous agents have been evaluated in clinical trials of this disease and criteria for assessment of efficacy are measurable. Most of the agents (non biologies) currently in use in the treatment of RA have side effects and toxicities that prevent either their long-term use or prevent dosing at levels that might provide superior disease modifying effects. The animal models often predict this phenomenon in that excellent efficacy can be achieved

at high doses but prolonged dosing at those levels results in serious toxicity in the animals. Generally, the effective dose 40-50 dose levels are safe for prolonged dosing periods in animals but these doses result in mostly moderate anti-inflammatory effects and modest, if any, beneficial effects on cartilage and bone lesions. In comparison to the osteo-arthritis models, RA models are relatively easy to perform, have good reproducibility of data and are generally of short duration. Most of the RA models have some pathological features that are similar to those occurring in human disease. Important differences include 1) animal models of RA progress much more rapidly than does human disease and thus are characterized by primarily acute inflammatory responses and 2) rodents have a tendency to have marked bone resorption and bone formation (especially periosteal/endosteal) in response to joint inflammation. The use of animal models of RA has contributed greatly to the overall knowledge of processes/mediators important in the generation of inflammation, cartilage destruction and bone resorption, thus leading to important advances in therapeutic intervention in this destructive disease. The focus of this paper will be on the models commonly used for screening/testing of potential pharmaceutical agents with less detailed discussion of the less utilized transgenic animal models. The approach I took when preparing this perspective on RA models was to try to provide the information that is

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commonly requested from me when I go to various pharmaceutical companies or academic institutions and am asked questions about research in RA and relevant (to human disease) models. In this paper, I've covered all the models that are commonly used for pharmaceutical testing in RA and placed emphasis on the ones that are considered to be most useful for testing the types of agents that are currently being investigated.

Adjuvant Arthritis

Rat adjuvant arthritis is an experimental model of polyarthritis which has been widely used for preclinical testing of numerous anti-arthritic agents which are either under preclinical or clinical investigation or are currently used as therapeutics in this disease[1-3]. The hallmarks of this model are reliable onset and progression (Figs. 1, 2) of robust, easily measureable, polyarticular inflammation, marked bone resorption and periosteal bone proliferation. Cartilage destruction occurs but is disproportionally mild in comparison to the inflammation and bone destruction that occurs. The pathogenesis/reasons for development of adjuvant disease following injection of arthrogenic preparations are not fully understood despite the fact that numerous studies have contributed to the understanding of various possibilities including reactivity to cartilage proteoglycans, heat shock proteins and interactions with intestinal flora[4-6]. Male Lewis rats (165-200 grams, 7/group) are generally used in studies of adjuvant arthritis. The disease develops in females but is much more variable in onset and severity. Animals should be allowed to acclimate for at least 3 days prior to initiation of experimentation. Induction of adjuvant disease can be done with either Freunds complete (FCA) supplemented with mycobacterium or by injection of the synthetic adjuvant N, N-dioctylddecyl-N', N-bis(2-hydroxy-ethyl) propanediamine (LA)[7]. Adjuvant can be injected at the base of the tail or in one of the foot pads. If injection is into the footpad, it allows study of the acute inflammatory reaction in that local area as well as the immunological reaction that develops approximately 9 days later in the contralateral paw and various organs. Hind paw swelling is monitored from day 9 (onset of disease) to 15 or greater depending on duration desired. In the later stages of disease (day 12+), adjuvant arthritis rats are often relatively immobile due to severity of paw swelling and so require special care to insure that they have access to water and food. To assess disease progression, caliper measurements of ankle joint width or volume using a water displacement device are done prior to the onset of arthritis, and then every other day until the study is terminated on day 15 post injection of the adjuvant. Clinical evidence of arthritis occurs on day 9-10 post injection of adjuvant. Treatments are initiated on day 0 (prophylactic model dosing) or day 8 (therapeutic model). Various stress-related factors including manipulations during the test period (pharmacokinetic sampling), frequency of dosing (QD vs BID) or type of vehicle used can influence the disease progression. However, once a certain methodology is established, controls over various studies will usually progress quite similarly (Fig. 3).

At termination, the tibiotarsal joint is transected at the level of the medial and lateral malleolus for determination of paw weights as another measure of inflammation or its inhibition. Paws are then collected into formalin for histopathological evaluation for beneficial effects on arthritis parameters and also for evaluation of potential deleterious effects of treatment on bone marrow. Obviously a clinically effective inhibitor that results in bone marrow hypocellularity as a mechanism of action would not be a desirable therapeutic agent in RA patients.

Ankle joints (with digits removed) are collected into 10% neutral buffered formalin for at least 24 hours prior to placement in Surgipath decalcifier I (Grayslake, IL.) for
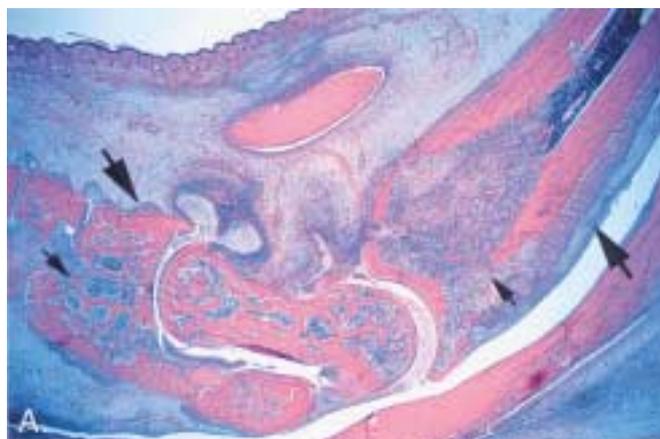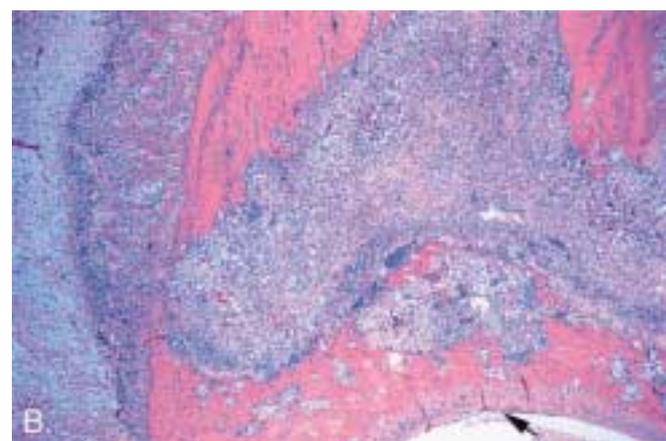

Figure 1a. Photomicrograph of ankle joint from adjuvant arthritic rat 15 days post-adjuvant injection at the base of the tail. Severe periarticular inflammation with marked edema is present with areas of bone resorption (thin arrows) as well as periosteal proliferation (thick arrows) (Hematoxylin and eosin, original magnification= 16X).

Figure 1b. Higher magnification of tibiotarsal area showing bone/growth plate resorption in association with numerous osteoclasts in an area where subchondral bone has largely been resorbed but cartilage (arrow) is largely unaffected. Hematopoietic/ adipose marrow has been replaced by marrow composed of mesenchymal cells with embedded inflammatory cells (Hematoxylin and eosin, original magnification= 100X).

approximately 1 week. When decalcification is complete, the ankle joint is transected in the longitudinal plane to give approximately equal halves. Joints are processed for paraffin embedding, sectioned and stained with hematoxylin and eosin for general evaluation and stained with toluidine blue for specific evaluation of cartilage changes if desired. Multiple sections are prepared (if necessary) to ensure that the distal tibia is present with both cortices and abundant distal tibial medullary space available for evaluation. Adjuvant arthritic ankles are given scores of 0-5 for inflammation and bone resorption as previously described[8].

Cartilage damage may or may not be scored in the adjuvant model. We have generally found this to be a minor feature and therefore not reliable for evaluation of potential treatment effects.

Use of the adjuvant model offers an opportunity to study pathological changes in a variety of tissues other than the joints and to develop knowledge of profiles of activity of various types of agents. Particularly useful is the splenomegaly that occurs in this model as certain types of agents (prostaglandin inhibitors) have no beneficial effects on this parameter while reducing paw swelling[3]. Splenomegaly occurs as a result of profound induction of extramedullary hematopoiesis in the red pulp in conjunction with pyogranulomatous inflammation in the red pulp and capsule. These changes are usually in association with mild to marked lymphoid atrophy. Ideally, an agent active in adjuvant disease should restore the spleen weights and morphology to normal as is the case with methotrexate treatment[8].

Hepatomegaly also occurs as a result of hypertrophy of hepatocytes and should be beneficially affected by treatment. Also fairly consistently present in these animals is an anterior uveitis which may be histologically evaluated for treatment effects.

Clinically used agents that are active in adjuvant arthritis include corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and low dose methotrexate[8]. The newer biologic agents such as the interleukin-1 receptor antagonist (IL-1ra) and soluble TNF receptors also have activity in this model[9-12]. Demonstration of efficacy with IL-1ra is dependent on maintaining sufficient blood levels for prolonged receptor antagonism either by continuous infusion methodologies or by the use of slow release vehicles[9]. Inhibition of IL-1 in the adjuvant model dramatically inhibits the bone resorption that is a prominent feature of this disease but has little effect on the inflammation. Studies such as these with specific inhibitors used under optimal pharmacokinetic conditions have helped delineate the importance of various cytokines in disease progression in adjuvant disease. Combination therapies, a likely clinical scenario, in this model using IL-1ra and methotrexate have shown the potential for additive effects[10]. Combinations may allow the reduction in dose of more toxic agents such as methotrexate while achieving superior efficacy than could be achieved with either agent alone.

The soluble TNF receptors have also been evaluated for efficacy in this model. A pegylated version of the type I receptor (PEG sTNF-RI) is active against both inflammation and bone resorption[11] and shows additive benefit when used in combination with traditional agents like methotrexate[13] and dexamethasone[12] as well as in combination with IL-1ra[14].

Rat Type II Collagen Arthritis

Rat type II collagen arthritis results when rats are immunized against homologous or heterologous type II collagen. The resulting polyarthritis is characterized by marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption and periosteal proliferation, and moderate to marked synovitis
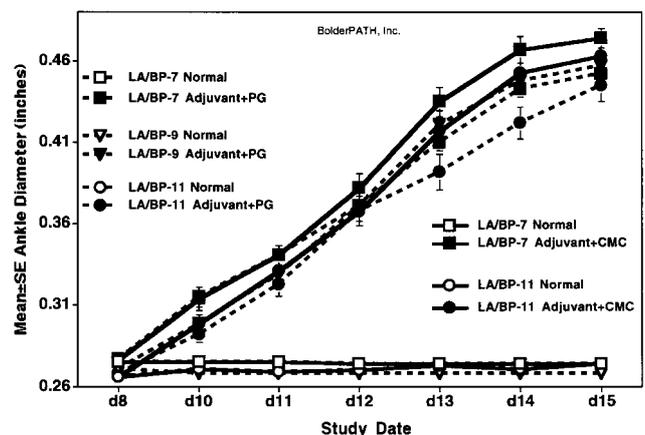

Figure 2. Photomicrograph of ankle joint from adjuvant arthritic rat 6 months post-adjuvant injection at the base of the tail. Severe bone destruction/proliferation with remodeling has resulted in an ankle morphology that is severely distorted. However, despite the extensive bone changes, the articular cartilages (arrows) are relatively unaffected (Toluidine blue, original magnifications 6X).

Figure 3. Ankle diameter over time in adjuvant arthritic rats treated with 1% carboxymethylcellulose or propylene glycol from day 0-14. Note consistency of response over various tests using 2 different vehicles. Slight differences warrant the use of an appropriate vehicle control for every test article.

and periarticular inflammation[15,16]. The lesions in type II collagen arthritis are somewhat more analogous to those seen in human RA than are the lesions of adjuvant arthritis (Fig. 4) in that there is more extensive pannus associated cartilage destruction. However, adjuvant arthritis has been used much more extensively for pharmaceutical testing and therefore more data exists for comparison in humans.

Female rats (8/group) are given id/sc injections of bovine type II collagen (2 mg/ml in incomplete Freund's Adjuvant) at the base of the tail and over the back in 3 sites (250μl divided) on day 0 and day 7. Onset of arthritis occurs on days 10-13 and as rats develop the disease they are randomized to study groups and treatment is initiated. Rats are given 6 daily treatments and then killed on day 7 of arthritis for histopathological evaluation[17].

Caliper measurements of ankle joint width are done prior to onset of arthritis, on the day of randomization and on each subsequent study day until termination of the study on arthritis day 7. At termination, the tibiotarsal joint is transected at the level of the medial and lateral malleolus for determination of paw weights as another measure of inflammation. Paws and knees are collected into formalin for histopathological evaluation using a scoring system similar to that described for adjuvant arthritis[8].

Clinically used agents that show activity in established collagen arthritis include corticosteroids, indomethacin and to a lesser extent methotrexate[8]. Because of the short duration of testing in this model (vs adjuvant arthritis) higher, and generally marrow suppressive doses are required to demonstrate efficacy of methotrexate in this model. Biological agents such as IL-1ra and the soluble TNF receptors are active in this test system and combination therapies with IL-1 ra and PEG sTNF-RI show potential for greater than additive effects[14].

Other models of rheumatoid arthritis

Mouse Type II Collagen Arthritis

Mice (DBA/1 lacJ) reliably develop polyarthritis when immunized against bovine type II collagen using a variety of methodologies including day 0/day 21 immunizations with and without concurrent boosting with endotoxin or recombinant IL-1. The disease that occurs is usually not symmetrical and any combination of paws/joints may be affected. Since caliper measurement of small mouse ankles is challenging, subjective clinical scoring systems are often used in conjunction with histological scoring methods. Treatments can be prophylactic (generally starting on day 21) or therapeutic (after observation of lesions) and depending on the immunization protocol used and extent of destruction desired, can extend for 10 days to several weeks. Lesions in affected joints resemble those occurring in rat collagen arthritis. This model has been particularly useful in evaluating the effects of biological agents such as IL-1ra and the soluble TNF receptors[18-22]. Enhancement of disease incidence and severity has been demonstrated in mice immunized with type II collagen and concurrently given cytokines such as IL-1[23,24].

Antigen Arthritis

Virtually any animal species can be used in the conduct of antigen arthritis studies. The animal of choice is immunized (subcutaneous or intradermal injections) with the antigen (usually a cationic substance such as methylated bovine serum albumin (m-BSA) which will bind to negatively charged cartilage and be retained in the joint). The antigen is then injected into one or both joints and acute inflammation progressing fairly rapidly to joint destruction ensues. The pathogenesis involves an Arthus reaction on the articular cartilage as antibodies to the positively charged antigen that is
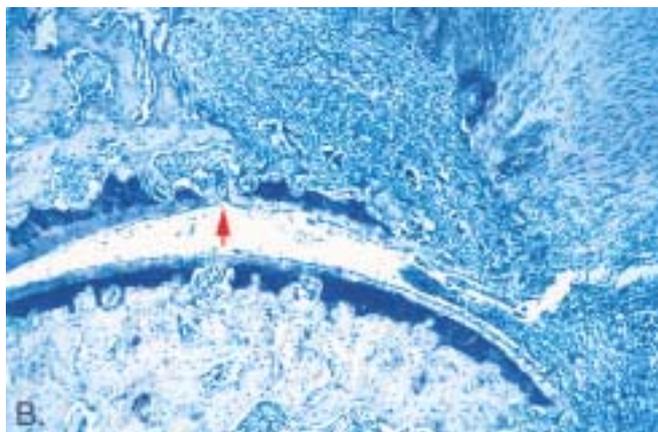

Figure 4a. Photomicrograph of ankle joint from type II collagen arthritic rat 7 days post-initial observation of swelling. Moderate periarticular inflammation with edema is present with areas of bone resorption (thin arrows) as well as periosteal proliferation (thick arrows). In contrast to the adjuvant model, cartilage destruction is present in most joints (arrow head) (Toluidine blue, original magnification= 16X).

Figure 4b. Higher magnification of tibiotarsal area showing bone resorption in association with numerous osteoclasts in an area where subchondral bone has largely been resorbed and cartilage (arrow) destruction is present in association with inflammatory cell infiltrate (Toluidine blue, original magnification= 100X).

injected form complexes that activate complement locally and result in cartilage destruction. Mouse models of antigen arthritis have been used extensively to study efficacy of biologies and the role of specific cytokines in the various aspects of disease pathogenesis[25-30]. The rabbit model of antigen arthritis is particularly useful when protocols require use of a larger joint[31]. Guinea pigs reliably develop antigen arthritis when immunized twice at 1 week intervals with m-BSA in Freund's complete adjuvant and are then injected intra-articulary with 300 pg of m-BSA, 3 weeks after the first injection. The model can be utilized as a combination prophylactic/therapeutic test using the following method. Three weeks post-initial immunization, inject (day 0) the right knee with m-BSA taking care not to inject the popliteal artery at the posterior aspect of the joint. Guinea pigs routinely develop antibodies which will cause acute death due to anaphylaxis if the antigen is administered systemically. An injection given too deep into the joint space will sometimes connect with this vessel and the result will be instantly apparent. A successful injection into the joint in an optimally immunized guinea pig will result in profound acute swelling within 6 hrs post-injection and this will be sustained at 24 (day 1) and 48 (day 2) hours. This allows identification of optimally sensitized animals and treatment can be initiated on day 1 or 2. On day 3 (animals have now been treated for 2 days), inject the contralateral knee to evaluate the effects of the treatment prophylactically. The lesions of antigen arthritis (Fig. 5a) progress extremely rapidly and in general greater effects of treatments will be observed using the prophylactic scenario. But this scenario is best not used alone unless some sort of preliminary testing is done to identify sensitized animals as up to 10% of a group may not be responders to the immunization. Agents active in human RA (cyclosporin, NSAIDs etc) are active on the prophylactic version of the model. Guinea pigs are insensitive to the action of corticosteroids, so they cannot be used as positive control
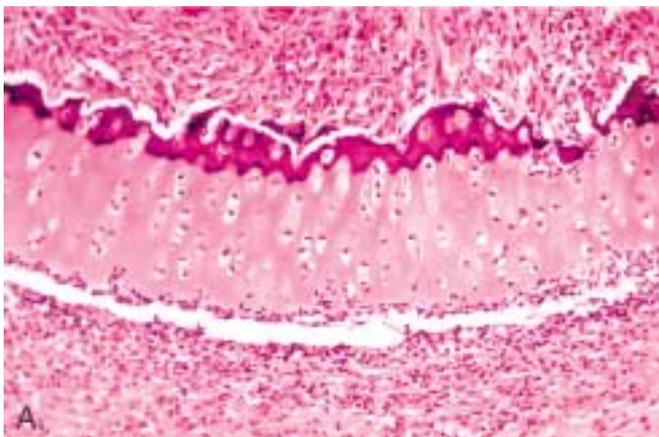
agents[32]. If the disease is allowed to progress for 2 weeks, histopathological evaluation reveals a highly destructive pannus which has destroyed most of the articular cartilage (Fig. 5).

Miscellaneous other rheumatoid arthritis models

Injection of aqueous suspensions of killed group A streptococci or peptidoglycans of their cell walls injected ip into susceptible rats (generally Lewis females are used) results in a biphasic polyarthritis with histological features that are similar to those of adjuvant arthritis[33]. Localized arthritic lesions can also be induced by intra-articular injection of streptococcal cell wall fragments with subsequent (18-25 days later) reactivation of inflammation via systemic administration of cell walls[34]. These models have been used to study the activity of various types of agents such as IL-1ra[35]. In general, the pathology in this model (either method of induction) resembles that of adjuvant arthritis in that there is abundant bone destruction, periosteal new bone formation and depending on the protocol used, varying degrees of cartilage destruction in association with the inflammatory process.

Several mouse strains develop RA-like lesions as a result of genetic manipulations. MRL/lpr mice sporadically develop immune complex polyarthritis, in association with their lymphoproliferative disorder, the incidence and severity of which is enhanced by administration of cytokines such as IL-1[36]. Without the added cytokine enhancement, the disease is too mild and variable in incidence and onset to be useful in pharmaceutical testing or pathogenesis studies.

There are 2 transgenic mouse models that have 100% incidence of inflammatory and destructive lesions resembling those occurring in RA.

Mice in which the TNF-α gene has been eliminated, transfected with a TNF-α gene that lacks the region necessary for cleavage of membrane bound TNF to soluble TNF, over express membrane bound TNF[35]. These animals

Figure 5a. Photomicrograph of medial knee joint from antigen arthritic guinea pig 7 days post-antigen injection into the knee. Numerous neutrophils are positioned on the surface of partially degraded articular cartilage and resorption of subchondral bone extends to calcified cartilage (Hematoxylin and eosin, original magnification= 100X).
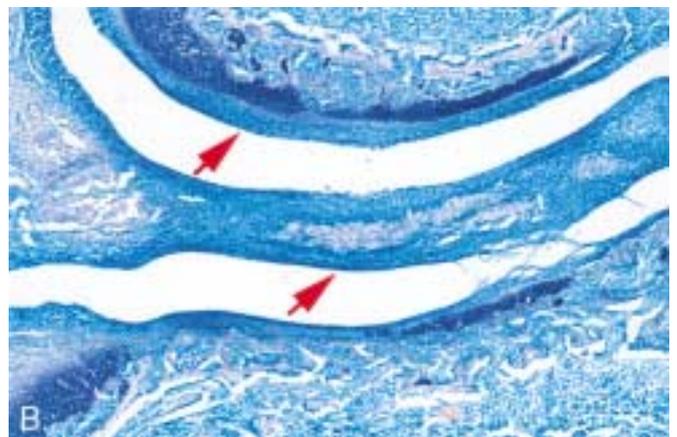

Figure 5b. Photomicrograph of medial knee 21 days post-injection of antigen showing residual attenuated articular cartilage and meniscus with surface fibrovascular pannus (arrows) (Toluidine blue, original magnification=25X).

reliably develop a deforming arthritis in the fore and hind paws beginning at 3 weeks of age. The lesions consist of periarticular inflammation (sometimes in nodular arrangement), bone resorption and retention of calcified cartilage/bone in the metaphysis and medullary cavities (Fig. 6). The soluble TNF receptors (both I and II) are active in inhibiting the disease process and some modulation is seen in animals treated with corticosteroids[37-39].

Other transgenic rodent models such as HLA-B27 transgenic rats[40], and B2-microglobulin-deficient mice lacking expression of HLA-B27[41] or having HLA-B27[42,43] develop spondylarthropathies of varying incidence and severity. These types of models have been used to study the importance of the major histocompatibility complex (MHC) class I proteins in the pathogenesis of inflammatory arthritis.

Mice transgenic for the V_B6 T cell receptor (TCR) (called KRN) crossed with NOD mice reliably develop rheumatoid arthritis-like lesions at about 27 days of age[44] as a result of the chance recognition of a NOD-derived major histocompatibility complex class II molecule by the transgenic TCR. The arthritis is chronic aggressive, bilaterally symmetrical, erosive poly-arthritis with joint destruction and clinical deformation. Effects of angiogenesis inhibition on synovial neovascularization demonstrated disease-modifying activities when treatment was started early in the disease progression[45].

Discussion

Ultimate selection of an animal model for studies on pathogenesis or effects of inhibitors of RA requires consideration of the purpose of the study. If the need is for rapid generation of preclinical efficacy/toxicity data to facilitate entry into clinical trials, selection of one of the induced (adjuvant, type II collagen) models is probably most appropriate. Generation of efficacy data in one of these models is procedurally straightforward and therefore should be reproducible. Test animals are readily available should the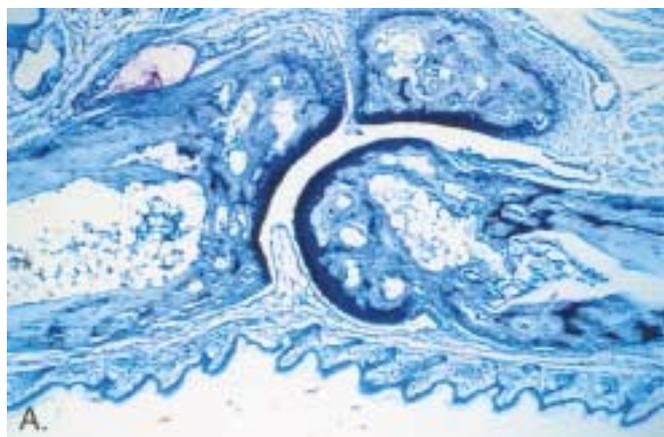 need for large-scale testing emerge. In addition, these models (adjuvant and collagen arthritis) have excellent track records for predicting activity and toxicity (at high doses of various agents) in humans. So comparative studies between older vs newer anti-arthritics can be done. Also, since these models are highly reproducible, examination of structure activity relationships between various molecules should be easily achievable.

Activity of commonly used small molecule anti-arthritic agents such as dexamethasone, indomethacin (and other NSAIDs including cyclo-oxygenase 2 inhibitors) and metho-trexate (adjuvant only) are predicted by the developing rat adjuvant and established rat type II collagen arthritis models.

Dexamethasone and other corticosteroids are used in the clinical treatment of RA but only at low doses[46] because of the toxicities associated with chronic use. Both animal models predict that corticosteroids have the potential to beneficially affect all aspects of RA and that they have the potential to be disease modifying but that toxicities associated with chronic dosing preclude their use at these efficacious doses. So the models predict that only modest clinical responses could be expected with a non-toxic dosing regime.

Indomethacin (and other NSAIDs) are active in both models. Efficacy peaks at 70% inhibition of AUC for paw swelling when daily oral doses of 1-2 mg/kg are given[8]. Inhibition of bone resorption in the adjuvant model also peaks at about 70% at these same doses. Other studies in which rats were dosed for longer periods of time with doses of 4 mg/kg/day have demonstrated the classic NSAID-induced lesions of intestinal and renal papillary necrosis (unpublished data, A. Bendele) thus demonstrating the narrow therapeutic index of this drug. More important however, is the suggestion from the animal bone resorption data that there is no dose that is likely to be profoundly disease modifying. Newer NSAIDs that are selective for cyclooxygenase 2 inhibition will
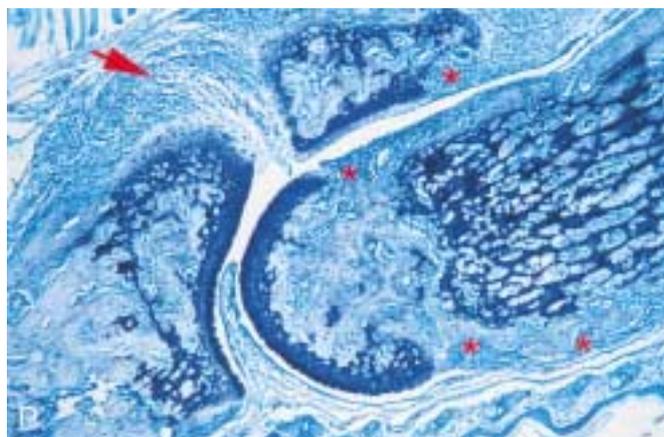

Figure 6a. Photomicrograph of metacarpophalangeal joint from normal mouse (Toluidine blue, original magnification=100X).

Figure 6b. Photomicrograph of metacarpophalangeal joint from TgA86 mouse over expressing membrane bound TNF. Synovium is thickened as a result of infiltration by inflammatory cells (arrow). Resorption of cortical bone (*) is evident in association with marked retention and filling of the medullary cavity with calcified cartilage and bone (Toluidine blue, original magnification= 25X).

likely eliminate the toxicities of the old cyclooxygenase 1 and 2 inhibitors but it is uncertain what their capacity for disease modification will be. In contrast to results in the adjuvant model, indomethacin showed excellent capacity to inhibit all aspects of rat type II collagen arthritis. Doses of 1-3 mg/kg/day resulted in 50-90% inhibition of clinical as well as histological parameters. So, this model predicts that indomethacin has the capacity to be a disease modifying agent but that prolonged dosing at these levels would result in unacceptable toxicity in the clinical setting. Generally, currently used NSAIDs are regarded as good anti-inflammatory therapy with little potential for disease modification at the doses that are generally safe for prolonged use in humans[47]. Both animal models predicted this and it will be interesting to see if the cyclooxygenase 2 inhibitors have disease modifying activity similar to that seen in rat type II collagen arthritis.

Methotrexate (low dose) has been one of the most successfully used anti-rheumatic agents[48,49]. It is most active in the developing adjuvant model where the opportunity exists to dose it for a longer period (15 days). The ED50 doses are generally 0.06 - 0.075 mg/kg/day. Other studies in which rats were dosed for longer periods of time have demonstrated bone marrow hypocellularity, intestinal lesions and death at doses of 0.1 mg/kg/day (unpublished data, A. Bendele). So, at 1-2 times the moderately effective dose, serious life-threatening toxicity occurs. A dose of 0.1 mg/kg/day can be given for 14 days in this model with the result being complete suppression of disease, thus demonstrating that this agent has the potential to provide disease modifying effects. Therefore, both models predict that methotrexate has the potential to be effective on all aspects of disease but that doses resulting in really dramatic efficacy would not be tolerated long-term. Although many patients have had excellent responses to methotrexate therapy, there is still room for improvement, especially in documentation of disease modification.

The biological agents (cytokine inhibitors), IL-1ra and soluble TNF-R2 (Enbrel) that are currently marketed for treatment of RA, are active in both rat and mouse animal models of arthritis with little or no evidence of toxicity. Likewise, current clinical trials with these agents have demonstrated efficacy and very little toxicity[50-52]. Conclusive clinical data on parameters indicative of disease modification will be needed before definitive statements can be made about the predictability of the animal data for disease modification.

Some of the newer transgenic models have interesting potential for use in delineating factors important in the pathogenesis of RA.

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